introduced at a location of *wild type Aequorea* GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of *wild type Aequorea* GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 *of wild type Aequorea GFP* and both of the aforementioned locations.

Claims 27 and 29 have been amended to recite an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence, wherein the modified GFP is derived from a wild type Aequorea GFP which has been mutated to further tailor sensitivity of the GFP (*e.g.,* the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro)) and operatively linked to expression control sequences, wherein the modified GFP sequence comprises LEPRAS (SEQ ID NO: 1) which has been introduced into one or more loops present on the surface of the GFP.

Support for the amendments can be found, for example, on page 1, line 18; page 8, lines 16-25 and page 10, lines 7-9 of the specification.

Supplemental Information Disclosure Statement (IDS)

Applicants' Attorney directs the Examiner's attention to the Supplemental IDS being filed herewith.

Objection to the specification

Under the section entitled "OBJECTIONS WITHDRAWN" in the Office Action, the Examiner states that the "use of trademarks has been noted in this application" and that they "should be capitalized wherever they appear and be accompanied by the generic terminology" (Office Action, page 3). The Examiner notes that "*Applicant has amended the specification to capitalize trademarks*", however, further states that the "*objection is maintained*" (Office Action, page 3).

In the telephonic interview on January 16, 2003, the Examiner stated that the objection had been withdrawn.

Rejection of Claims 11 and 14 under 35 U.S.C. §112, second paragraph

Claims 11 and 14 are rejected under 35 U.S.C. § 112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 3).

The Examiner states that "Claims 11 and 14 are not clear as to what end of the nucleic acid is the reference point from which to start counting from and to determine the position of where to introduce the restriction nuclease sites" and suggests "that the actual sequence be recited in the claims for clarifying the instantly claimed structure" (Office Action, page 6).

Applicants respectfully disagree. As amended, Claims 11 and 14 relate to an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea GFP nucleic acid sequence wherein the GFP is mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, and wherein the modified GFP sequence encodes a recombinant peptide which comprises restriction endonuclease sites, or encodes a heterologous amino acid sequence, introduced at a location of *wild type Aequorea* GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of *wild type Aequorea* GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 *of wild type Aequorea GFP* and both of the aforementioned locations, thereby producing a modified GFP. The sequence of wild type Aequorea GFP was known to those of skill in the art at the time of Applicants' invention. For example, Applicants direct the Examiner's attention to Prasher *et al., Gene, 111(2):*229-233 (1992) and WO 96/23810 (page 3, lines 3-4 and pages 21-22, SEQ ID NO: 2), which are listed in the Supplemental IDS being field concurrently. As indicated in Prasher *et al.* and WO 96/23810, the sequence of wild type GFP was known as early as 1992. *

The Examiner states that "Claim 11 is also confusing with respect to the phrase 'restriction endonuclease sites introduced'" (Office Action, page 6). The Examiner states that the "phrase in question suggest insertion of the nucleic acid sequence recognized by restriction enzymes", and "[i]f so it is not clear what nucleic acid sequences are being inserted" (Office Action, page 6). The Examiner suggests that "the actual sequence including the cleaved sites be recited in the claims for clarifying the instantly claimed structure and obviate the rejection" (Office Action, page 6).

Applicants respectfully disagree. Evidence that the phrase is ***not*** confusing is provided by the Examiner's understanding that the phrase indicates "insertion of the nucleic acid sequence recognized by restriction enzymes" (Office Action, page 6). Numerous restriction endonuclease sites are known and routinely used by those of skill in the art to facilitate insertion of heterologous DNA into a nucleotide sequence (*e.g.*, a plasmid, a vector), thereby producing an expression cassette. To amend the claim to recite one of the many known restriction endonuclease sites would unduly limit Applicants' claimed invention and would allow those of skill in the art to easily design around Applicants' invention.

Claims 11 and 14, particularly as amended, particularly point out and distinctly claim the subject matter which Applicants regard as the invention

Rejection of Claims 11, 14, 27 and 29 under 35 U.S.C. §112, first paragraph

Claims 11, 14, 27 and 29 are rejected under 35 U.S.C. §112, first paragraph "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention" (Office Action, page 6). The Examiner states that the "written description does not set forth any and all possible mutations (detections and/or substitutions) to the GFP nucleic acid of the instsn [sic] claims therefore the written description is not commensurate in scope with the claims" (Office Action, page 6).

Applicants respectfully disagree. To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention (*Vas-Cath, Inc. v. Mahurkar*, 19 U.S.P.Q.2d 1111,1116 (Fed. Cir. 1991)). As recited in the MPEP:

> The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed (see, e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993)) and ***should include a determination of the field of the invention and the level of skill and knowledge in the art***. Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. ***Information***

which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986) (MPEP, 8th edition, page 2100-160, emphasis added).

As amended, Claims 11 and 14 relates to an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea GFP nucleic acid sequence wherein the GFP is mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, and wherein the modified GFP sequence encodes a recombinant peptide which comprises restriction endonuclease sites, or encodes a heterologous amino acid sequence, introduced at a location of *wild type Aequorea* GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of *wild type Aequorea* GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 *of wild type Aequorea GFP* and both of the aforementioned locations, thereby producing a modified GFP. As amended, Claims 27 and 29 relate to an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence, wherein the modified GFP is derived from a *wild type Aequorea* GFP which has been mutated to further tailor sensitivity of the GFP (*e.g.*, the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro)) and operatively linked to expression control sequences, wherein the modified GFP sequence comprises LEPRAS (SEQ ID NO: 1) which has been introduced into one or more loops present on the surface of the GFP.

As pointed out above, at the time of Applicants' invention, the sequence of wild type Aequorea GFP was known to those of skill in the art (see Prasher *et al.* and WO 96/23810 cited in the Supplemental IDS). Aequorea GFP sequences which have been mutated to further tailor sensitivity of the GFP were also known at the time of Applicant's invention to those of skill in the art. For example, in the specification as filed, Applicants teach that:

> The magnitude of the spectral change, and thus the sensitivity of the biosensor, can be further tailored by introducing additional mutations, such as point mutations into the fluorescent protein amino acid sequence. For example, it has been reported that a mutation at position 147 from serine to proline facilitates protein folding, and thus the formation of the GFP chromophore (Ser65-Tyr66-Gly67). Alternatively, the modifications to introduce a heterologous amino acid sequence into a GFP protein described herein, can be introduced into a GFP

> mutant which has been genetically engineered to confer particular spectral properties to the starting protein. For example, United States Patent 5,804,387 describes three GFP mutants suitable for use as starting proteins for the production of aFP described herein. GFPmut1 has a double substitution: F64L, S65T; GFPmut2 has a triple substitution: S65A, V68L, S72A; and GFPmut3 is characterized by the double substitution S65G, S72A. The commercial availability of cloning vectors comprising the nucleotide sequences encoding these various forms of GFP facilitate the design, production and expression of aFP. For example, the cloning vector pEGFP (Clontech Catalog #6077-12) encodes the GFPmut1 variant which produces a modified red-shifted variant of wild-type green fluorescent protein which has been optimized for brighter fluorescence and higher expression in mammalian cells (specification, page 10, lines 7-24).

U.S. Patent No. 5,804,387 (the '387 patent), which is being filed in the Supplemental IDS being filed concurrently, describes numerous mutants of Aequorea GFP sequences which have been mutated to further tailor its sensitivity and which were known at the time of Applicants' invention (*e.g.*, the '387 patent, column 2, lines 17-48; column 3, lines 1-32). Further examples of Aequorea GFP mutants which were known at the time of Applicants' invention are described in WO 96/23810 (*e.g.*, page 6, line 6 - page 9, line 31).

Applicants' teaching in the specification regarding Aequorea GFP sequences which have been mutated to further tailor sensitivity of the GFP clearly demonstrate that Applicants had possession of the full scope of the claimed invention. Furthermore, such sequences were well known in the art at the time of Applicants' invention, and thus, it is not required that they be described in detail in the specification. Indeed, the court in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* stated that "a patent need not teach, ***and preferably omits***, what is well known in the art" (*Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986)).

* where no seq. identified with respect to the justification

Applicants' claimed invention clearly contains subject matter which was described in the specification in such a way as to reasonably convey to one skilled in the relevant art that Applicants, at the time the application was filed, had possession of the claimed invention

Rejection of Claims 11, 41, 27 and 29 under 35 U.S.C. §112, first paragraph

Claims 11, 14, 27 and 29 are rejected under 35 U.S.C. §112, first paragraph "because the specification does not reasonably provide description of or enablement for any and every

modified GFP nucleic acid sequence which is mutated" (Office Action, page 8). The Examiner states that "the disclosure does not provide guidance as to all modifications or structures encompassed by the broad claims" (Office Action, page 8). The Examiner further states that "an enabling disclosure for the preparation and use of only a few analogs of a product does not enable all possible analogs where the characteristics of the analogs are unpredictable" (Office Action, page 9).

Applicants respectfully disagree. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation" (*United States v. Teletronics, Inc.* 8 U.S.P.Q.2d, 1217, 1223 (Fed. Cir. 1988)). As pointed out above, the court in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* stated that "a patent need not teach, ***and preferably omits***, what is well known in the art" (*Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986)).

As amended, Claims 11 and 14 relate to an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea GFP nucleic acid sequence wherein the GFP is mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, and wherein the modified GFP sequence encodes a recombinant peptide which comprises restriction endonuclease sites, or encodes a heterologous amino acid sequence, introduced at a location of *wild type Aequorea* GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of *wild type Aequorea* GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 *of wild type Aequorea GFP* and both of the aforementioned locations, thereby producing a modified GFP. As amended, Claims 27 and 29 relate to an isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence, wherein the modified GFP is derived from a *wild type Aequorea* GFP which has been mutated to further tailor sensitivity of the GFP (*e.g.*, the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro)) and operatively linked to expression control sequences, wherein the modified GFP sequence comprises LEPRAS (SEQ ID NO: 1) which has been introduced into one or more loops present on the surface of the GFP.

In the specification as filed, Applicants teach that:

> The magnitude of the spectral change, and thus the sensitivity of the biosensor, can be further tailored by introducing additional mutations, such as point mutations into the fluorescent protein amino acid sequence. For example, it has been reported that a mutation at position 147 from serine to proline facilitates protein folding, and thus the formation of the GFP chromophore (Ser65-Tyr66-Gly67). Alternatively, the modifications to introduce a heterologous amino acid sequence into a GFP protein described herein, can be introduced into a GFP mutant which has been genetically engineered to confer particular spectral properties to the starting protein. For example, United States Patent 5,804,387 describes three GFP mutants suitable for use as starting proteins for the production of aFP described herein. GFPmut1 has a double substitution: F64L, S65T; GFPmut2 has a triple substitution: S65A, V68L, S72A; and GFPmut3 is characterized by the double substitution S65G, S72A. The commercial availability of cloning vectors comprising the nucleotide sequences encoding these various forms of GFP facilitate the design, production and expression of aFP. For example, the cloning vector pEGFP (Clontech Catalog #6077-12) encodes the GFPmut1 variant which produces a modified red-shifted variant of wild-type green fluorescent protein which has been optimized for brighter fluorescence and higher expression in mammalian cells (specification, page 10, lines 7-24).

U.S. Patent No. 5,804,387 (the '387 patent), which is cited in the Supplemental IDS being filed concurrently, describes numerous mutants of Aequorea GFP sequences which have been mutated to further tailor its sensitivity and which were known at the time of Applicants' invention (*e.g.*, the '387 patent, column 2, lines 17-48; column 3, lines 1-32). Further examples of Aequorea GFP mutants which were known at the time of Applicants' invention are described in WO 96/23810 (*e.g.*, page 6, line 6 - page 9, line 31). Thus, at the time of Applicants' invention, the sequence of wild type Aequorea GFP and numerous Aequorea GFP sequences which have been mutated to further tailor sensitivity of the GFP were known to those of skill in the art.

However inventive guidance BUT THOUGHT?

Clearly, one reasonably skilled in the art could make or use the claimed expression cassette comprising a modified GFP nucleic acid sequence wherein the GFP is mutated to further tailor sensitivity of the GFP using the guidance Applicants provide in the specification as filed coupled with information known in the art without undue experimentation. Applicants have provided an enabling disclosure for the full scope of the claimed invention.

Rejection of Claims 11, 13, 14 and 15 under 35 U.S.C. §103(a)

The rejection of Claims 11, 13, 14 and 15 under 35 U.S.C. §103(a) "as being unpatentable over Tsien et al. (WO 97/28261) in view of Tsien et al. (USC Patent #6,066,476) and further in view of Miesenbock et al. (WO 98/36081)" is maintained (Office Action, page 10).

Applicants respectfully disagree. As Applicants' Attorney pointed out in the telephonic interview, the combined teachings of the cited references do not teach or suggest introducing a recombinant peptide which comprises restriction endonuclease sites or a nucleotide sequence which encodes a heterologous amino acid sequence ***within*** a GFP molecule.

As amended, Applicants' claimed invention relates to an isolated affinity fluorescent protein expression cassette comprising ***a single*** modified Aequorea green fluorescent protein (GFP) nucleic acid sequence, wherein the modified GFP is derived from a *wild type Aequorea* GFP which has been mutated to further tailor sensitivity of the GFP (*e.g.*, the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro)) and operatively linked to expression control sequences, wherein the ***single*** modified GFP sequence comprises restriction endonuclease sites (*e.g.*, LEPRAS (SEQ ID NO: 1)) or a nucleotide sequence which encodes a heterologous amino acid sequence, which has been introduced into one or more loops present on the surface of ***a single*** GFP (*e.g.*, between glutamine (Gln) 157 and lysine (Lys)158 of *wild type Aequorea* GFP; between glutamic acid (Glu)172 and aspartic acid (Asp)173 *of wild type Aequorea GFP*; both of the aforementioned locations).

As pointed out in the previously filed Amendment and in the telephonic interview, Tsien *et al.* (WO 97/28261) teach "[t]andem fluorescent protein constructs in which ***two fluorescent protein moieties*** capable of exhibiting FRET [fluorescence resonance energy transfer] are coupled through a linker to form a tandem construct" (Tsien *et al.* WO/9728261; page10, line 32 - page 11, line 1, emphasis added). Tsien *et al.* (WO 97/28261) further teach that the "linker moiety is, preferably, a peptide moiety, but can be another organic molecular moiety" and that "[r]upture of the linker moiety results in separation of the fluorescent protein moieties that is measurable as a change in FRET" (Tsien *et al.* (WO 97/28261), page 23, lines 3-12). In a particular embodiment, the linker is a "cleavage site for a protease of interest" (Tsien *et al.* (WO 97/28261), page 12, lines 18-19). Tsien *et al.* (WO 97/28261) do not teach introducing a recombinant peptide which comprises restriction endonuclease sites or a nucleotide sequence that

encodes a heterologous amino acid sequence ***within a single GFP molecule***. Applicants direct the Examiner's attention to the Exhibit being filed concurrently. The Exhibit is a schematic illustrating the differences between Tsien *et al.* and Applicants' claimed invention.

Tsien *et al.* (U.S. Patent 6,066,476) teach that "particular modifications in the polypeptide sequence of an Aequorea wild type GFP . . . lead to formation of products having markedly different excitation and emission spectra from corresponding products derived from wild-type GFP" (Tsien *et al.* (U.S. Patent 6,066,476), column 2, lines 24-28). The modified GFP in Tsien *et al.* (U.S. Patent 6,066,476) have one or more point mutations and exhibit "significant alterations in the ratio of the two main excitation peaks" (Tsien *et al.* (U.S. Patent 6,066,476), column 3, lines 62-63), exhibit fluorescence "at different wavelengths" (Tsien *et al.*, (U.S. Patent 6,066,476), column 4, lines 53-54), or exhibit "substantially more intense fluorescence per molecule than the wild type molecule" (Tsien *et al.*, (U.S. Patent 6,066,476), column 5, lines 12-14). The Examiner states that "[s]everal different restriction endonuclease sites are discussed" (Office Action, page 10) in Tsien *et al.* (U.S. Patent 6,066,476), however, Applicants fail to find such a teaching. Tsien *et al.* (U.S. Patent 6,066,476) state that the modified GFP can be used as "reporter genes for monitoring the expression of sequences fused thereto"; "as a means to trace lineage of a gene fused thereto"; as "a genetic marker"; and as "a fluorescent tag" (Tsien *et al.*, column 6, lines 44-66). *However, Tsien et al. do not teach or even suggest that a restriction endonuclease site or a nucleotide sequence which encodes a heterologous amino acid sequence can be introduced* ***within*** *the modified GFP sequence.*

Miesenbock *et al.* teach "hybrid molecules comprising a targeting region and a reporter region capable of participating in a reaction resulting in an optically detectable signal when the hybrid molecule encounters a change in the microenvironment" and that a "linker comprising at least one amino acid may also be interposed between the targeting and reporter regions" (Miesenbock *et al.*, page 18, lines 2-5 and 20-21). The reporter region of the hybrid molecules "may be any molecular moiety that participates in a bioluminescent, chemiluminscent, fluorescent, or fluorogenic reaction", such as GFP, and preferably, the reporter "is an amino acid sequence which is co-expressed as a fusion protein with the targeting amino acid sequence" (Miesenbock *et al.*, page 23, lines 30-32; page 25, lines 11-13). In addition, Miesenbock *et al.* describe environment-sensitive GFP mutants, termed pHluorins, which were generated using

various amino acid substitutions shown in Table 2 (Miesenbock *et al.*, page 48). However, *Miesenbock et al. do not teach or even suggest that a restriction endonuclease site or a nucleotide sequence which encodes a heterologous amino acid sequence can be introduced* ***within*** *a modified GFP sequence.*

None of the cited references, either alone or in combination, teaches or suggests introducing a recombinant peptide which comprises restriction endonuclease sites or a nucleotide sequence which encodes a heterologous amino acid sequence ***within*** a GFP molecule. Tsien *et al.* (WO 97/28261) teach tandem fluorescent protein constructs in which ***two fluorescent protein moieties*** capable of exhibiting FRET are coupled through a linker. Tsien *et al.* (USC Patent # 6,066,476) and Miesenbock *et al.* discuss modified GFPs which include one or more amino acid substitutions, but do not further teach introducing any heterologous sequence ***within*** a GFP molecule.

Clearly, Tsien *et al.* (WO 97/28261) in view of Tsien *et al.* (U.S. Patent No. 6,066,476) and further in view of Miesenbock *et al.* (WO 98/36081) do not render obvious Applicants' claimed invention.

Rejection of Claims 12 and 27-29 under 35 U.S.C. §103(a)

Claims 12 and 27-29 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Tsien et al. (WO 97/28261) and Tsien et al. (USC Patent # 6,066,476) in view of Miesenbock et al. (WO 98/36081) and in further view of Gorman et al. (WO 99/19489)" (Office Action, page 11).

Applicants respectfully disagree. As pointed out above, the combined teachings of Tsien *et al.* (WO 97/28261) and Tsien *et al.* (USC Patent # 6,066,476) in view of Miesenbock *et al.* (WO 98/36081) do not teach or suggest introducing a recombinant peptide which comprises restriction endonuclease sites or a nucleotide sequence which encodes a heterologous amino acid sequence ***within*** a GFP molecule. Tsien *et al.* (WO 97/28261) teach tandem fluorescent protein constructs in which ***two fluorescent protein moieties*** capable of exhibiting FRET are coupled through a linker. Tsien *et al.* (USC Patent # 6,066,476) and Miesenbock *et al.* discuss modified GFPs which include one or more amino acid substitutions, but do not further teach introducing any heterologous sequence ***within*** a GFP molecule.

Gorman *et al.* teach "the amino acid sequences of a new CNGC [cyclic nucleotide gated channels] and Myosin that map to a region of the human chromosome associated with Bardet-Biedl Syndrome" (Gorman *et al.*, abstract). It appears as though the Examiner is stating that Applicants' LEPRAS is contained within SEQ ID NO: 1 of the Gorman *et al.* published application, however, the Examiner has not specifically pointed out where LEPRAS is within SEQ ID NO: 1. Applicants respectfully request that the Examiner provide the report showing that LEPRAS is contained within SEQ ID NO: 1 of the Gorman *et al.* published application if a rejection based on Gorman *et al.* is maintained. However, the rejection should be withdrawn because Gorman *et al.* do not discuss fluorescent proteins such as GFP, and thus, Gorman *et al.* clearly do not provide the teaching lacking in the combined teachings of the Tsien *et al.* (WO 97/28261), Tsien *et al.* (USC Patent # 6,066,476) and Miesenbock *et al.* references.

Tsien *et al.* (WO 97/28261) and Tsien *et al.* (U.S. Patent No. 6,066,476) in view of Miesenbock *et al.* (WO 98/36081) and in further view of Gorman *et al.* (WO 99/19489) do not render obvious Applicants' claimed invention.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,
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Dated: January 30, 2003

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

11. (Twice mended) An isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid DNA sequence [which], wherein the GFP is mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, and wherein the modified GFP sequence [comprises] encodes a recombinant peptide which comprises restriction endonuclease sites introduced at a location of [the] wild type Aequorea GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of wild type Aequorea GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 of wild type Aequorea GFP and both of the aforementioned locations.

14. (Twice Amended) An isolated affinity fluorescent protein expression vector comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence [which], wherein the GFP is mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, and wherein the modified GFP sequence [comprises] encodes a heterologous amino acid sequence introduced at a position of [the] wild type Aequorea GFP molecule selected from the group consisting of: between glutamine (Gln) 157 and lysine (Lys)158 of wild type Aequorea GFP, between glutamic acid (Glu)172 and aspartic acid (Asp)173 of wild type Aequorea GFP and both of the aforementioned locations.

27. (Amended) An isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence [which], wherein the modified GFP is derived from a wild type Aequorea GFP which has been mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a hexapeptide LEPRAS (SEQ ID NO: 1) which has been introduced into one or more loops present on the surface of the GFP.

29. (Amended) An isolated affinity fluorescent protein expression cassette comprising a modified Aequorea green fluorescent protein (GFP) nucleic acid sequence [which], wherein the modified GFP is derived from a wild type Aequorea GFP which has been mutated to further tailor sensitivity of the GFP and operatively linked to expression control sequences, wherein the modified GFP comprises a substitution of serine at position 147 of GFP to proline (Ser147Pro) and a hexapeptide LEPRAS (SEQ ID NO: 1) which has been introduced into one or more loops present on the surface of the GFP.